IS No.	Title
IS 13426 : 1992	Animal feeds and feeding stuffs — Methods of sampling for aflatoxin analysis
IS 13427 : 1992	Animal feeds and feeding stuffs — Determination of aflatoxin B1 content
IS 14718 : 1998	Animal feeding stuffs — Determination of aflatoxin B1 content of mixed feeding stuffs — Method using high performance liquid chromatography
IS 14826 : 2000/ISO 5985 : 1978	Animal feeding stuff — Determination of ash insoluble in hydrochloric acid

ANNEX B
(*Clause* 4.1.1)

INGREDIENTS FOR *PANGASIUS* FISH FEED

B-1 In the compounding of *Pangasius* fish feed a variety of ingredients are used. This Annex gives a list of such ingredients.

B-1.1 Ingredients of Animal Origin

a) Fishmeal (*see* IS 4307) and all other fish products;
b) All crustacean meals including prawn head meal, prawn shell meal, Acetes and Krill meal;
c) Squid meal and all other squid products;
d) Molluscan meal (clam, mussel, etc);
e) Fish solubles and hydrolysates;
f) Fish oil;
g) Squid oil;
h) Squid liver oil;
j) Chicken oil;
k) Insect meal and oils;
m) Silk worm Pupae meal;
n) Bone meal (*see* IS 1942);
p) Meat and Bone meal;
q) Poultry by product meal;
r) Blood meal;
s) Krill oil;
t) Tallow; and
u) Squid liver paste.

B-1.2 Ingredients of Plant Origin

a) Soybean cake (meal);
b) Groundnut oilcake (expeller-pressed or solvent extracted) (*see* IS 1713 and IS 3441);
c) Sesame (*Sesamum indicum* Linnaeus) oilcake (expeller-pressed or solvent extracted) (*see* IS 1934);
d) Cottonseed oilcake (decorticated) (expeller-pressed or solvent extracted) (*see* IS 1712 and IS 3592);
e) Sunflower oilcake (decorticated or undecorticated);
f) Copra cake, coconut oilcake (expeller-pressed or solvent extracted) (*see* IS 2154 and IS 3591);
g) Mustard oil cakes (*see* IS 1932);
h) Palm kernel meal;
j) Rapeseed oil cake;
k) Wheat and wheat products;
m) Rice and its products/broken rice;
n) Maize and maize products;
p) Any other edible cereal and its product;
q) Rice bran (*see* IS 3648);
r) Wheat bran;
s) Edible vegetable oils;
t) Soybean lecithin;
u) Algal meals and oils;
v) Sea weeds;
w) Corn gluten;
y) Wheat Gluten;
z) Dried distillery grains with solubles (DDGS);
aa) Detoxicated jatropha meal; and
bb) Detoxicated Mahua oil cake

B-1.3 Other Ingredients

a) Vitamins;
b) Minerals;
c) Common salt (*see* IS 920);
d) Dicalcium phosphate (*see* IS 5470), Mono Calcium Phosphate (*see* IS 5470), Mono sodium phosphate, Mono potassium phosphate, Mono ammonium phosphate;
e) Yeast and Yeast extracts;
f) Spirulina;
g) Brewery by-products;
h) Molasses (*see* IS 1162);
j) Tapioca and its products;
k) Binders;
m) Single cell protein;
n) Attractants;
p) Nucleotides;
q) Amino acids;
r) Pigments;
s) Toxin binders and Clay;
t) Dunaliella;
u) Antifungals;
v) Peptidoglycans;
w) ßglucans;
y) Fuccoidan;
z) Organic acids;
aa) Guar meal (*see* IS 4193); and
bb) Seaweed.

ANNEX C

(*Clause* 4.2.2)

DETERMINATION OF WATER STABILITY OF *PANGASIUS* FISH FEED PELLETS

C-1 PRINCIPLE

Water stability of dry ***Pangasius*** fish feed pellets is determined by the loss in mass of pellets kept in water for a specified time interval. The loss in mass of pellets indicates the stability, higher the loss poorer the stability.

C-2 APPARATUS

C-2.1 Oven

C-2.2 Nylon Mesh

C-2.3 Sieve (2.4 mm)

C-2.4 Balance

C-2.5 Glass Beaker (1 litre)

C-2.6 Stop Watch

C-3 PROCEDURE

Wash cone shaped pouches made of nylon mesh (1 mm mesh size) thoroughly and dry at 70 °C to constant mass in an oven. Take about 2 g of feed pellets in each pouch and record exact initial mass. Take 5-6 such pouches for each sample. Place the pouches with feed pellets at the bottom of 1 liters beaker containing one liters water. Record water temperature. After prescribed time, slowly take out pouches with pellets out of the water. Examine the pellets for their physical shape. Dry the pouches with pellets at 70 °C to constant mass. Difference in the initial mass and final mass of the pellets gives loss in mass at 70 °C.

C-4 CALCULATION

Water stability is calculated using the following formula:

$$\text{Percent Water Stability} = \frac{Final\ mass\ (g) \times Percent\ dry\ matter}{Initial\ mass\ (g) \times Percent\ dry\ matter} \times 100$$

ANNEX D

[*Table* 1, *Sl No.* (vi)]

GROSS ENERGY

Gross energy in raw materials and finished *Pangasius* fish feeds can be either directly estimated by bomb calorimeter (Method D-1) or calculated by Physiological Fuel Values (Method D-2) and the detailed procedures are given below.

D-1 DETERMINATION OF GROSS ENERGY

The bomb calorimeter provides a means of assessing the amount of energy (gross) made available during the catalytic degradation of combustible solids, liquids and gases in a pressurized oxygen atmosphere. Gross energy is the amount of heat liberated when a substance is completely burnt to carbon dioxide and water. It is also known as heat of combustion.

D-1.1 Preparation of Sample Material

It is essential that the test sample is truly representative of the sample material. In general, the sample material needs to be dried before combustion and here the sample characteristics will determine the method of drying to be used that is whether oven drying or vacuum drying at low temperature should be done before or after selection of a working sample. The drying process should not volatilize or destroy any of the combustible material. If complete dryness cannot be achieved easily without loss, preliminary tests should be made to determine the maximum water content at which this sample material can be ignited and completely burnt in the bomb. All material which have low bulk density and high surface area must be compacted.

D-2 PRINCIPLE

A known quantity of a sample is ignited electrically and burnt in excess of oxygen in the bomb. The maximum temperature rise is measured with the thermometers in a controlled system. By comparing this rise with that obtained when a sample of known calorific value is burnt, the calorific value of the sample material can be determined.

D-3 APPARATUS

D-3.1 Adiabatic Bomb Calorimeter

D-3.2 Pellet Press

D-3.3 Metallic Crucible

D-3.4 Hot Air Oven

D-3.5 Balance

D-3.6 Fuse Wire

D-3.7 Cotton Thread

D-3.8 Beaker

D-3.9 Burette

D-3.10 Pipette

D-3.11 Whatman Filter Paper No. 1

D-4 REAGENTS

D-4.1 Benzoic Acid (Calorimeter Grade, Gross Energy Content 6 318 cal/g)

D-4.2 Distilled Water

D-4.3 Oxygen Gas

D-4.4 Barium Hydroxide

D-4.5 Sodium Carbonate

D-4.6 Hydrochloric Acid

D-4.7 Methyl Red Indicator

D-4.8 Phenolphthalein Indicator

D-5 PROCEDURE

D-5.1 Determination of Bomb Equivalent

a) Take about 0.35 g of benzoic acid and make a pellet with the help of a pellet press;

b) Place the pellet in a pre-weighed metallic crucible. Weigh the pellet and crucible accurately;

c) Put the bomb top on the stand. Thread a piece of fuse wire through the electrodes and tie a single strand of cotton to it. Keep the lengths of fuse wire and cotton thread constant in order to facilitate the calculation of caloric value;

d) Swing the crucible into position, clamp the ring and arrange the ends of the cotton thread so that they are in contact with the sample;

e) Pipette 1 ml of distilled water into the bomb;

f) Place the electrode assembly into the bomb body ensuring that it fits correctly;

g) Tighten the bomb closure ring by hand only;

h) Fill the bomb to 25 atmospheric pressure with oxygen (oxygen must be free from hydrogen);

j) Fill water into calorimetric vessel to submerge the bomb completely. The vessel and water should give a total mass of 3 kg. The quantity of water used is not critical but it must be constant for all tests to an accuracy of ± 0.5 kg.

k) Place the bomb on three supports in the calorimeter vessel and check for the gas leakage that the bomb should not show any sign of gas leakage;

m) Gently slide the top of the calorimeter console onto the bomb. Switch on the main and press down the bomb firing plug to contact the bomb;

n) Adjust the initial temperature and press the fire switch; and

p) After 8 min read the temperature on main thermometer. Note final temperature when it stabilizes.

D-5.1.1 Calculation

$$\text{Bomb equivalent} = \frac{(6\,318 \times M) + A}{T}$$

where

M = Mass of benzoic acid (g);
A = Correction factor for wire and thread [heat of combustion of thread and wire may be taken as 3 962 cal/g and 1 400 cal/g (or 2.3 cal/cm) respectively]; and
T = Rise in temperature (°C).

D-5.2 Gross Energy Estimation of Feed

Weight 0.51 g of finely ground representative sample and make a pellet with the help of pellet press. All the materials which have low bulk density and high surface area must be compacted to reduce their rate of combustion, or otherwise, it will lead to false result due to loss of sample from the crucible, even more serious is the possibility that the combustion will be so rapid that it resembles an explosion. Weigh samples for dry matter determination at the time of pelleting. Put the pellet in a pre-weighed crucible and weigh again. Follow the steps (c) to (p) as described in **D- 5.1**. Switch off the main switch. Remove the bomb from the vessel. Release pressure of the bomb using pressure release cap. Open the bomb and wash the electrodes and inside top and body of the bomb with distilled water. Collect these washing in a beaker for corrections for nitrogen and sulphur contents.

D-5.2.1 Calculation

$$\text{Gross energy (cal/g)} = \frac{(\text{Bomb equivalent} \times T) - A}{\text{Dry mass of sample (g)}}$$

where

T = Rise in temperature; and
A = Correction factors for wire, thread, nitrogen and sulphur.

D-5.3 Nitrogen and Sulphur Corrections

a) Boil the washings (see **D-5.2**) collected in the beaker for 5 min;
b) Cool and titrate against N/10 $Ba(OH)_2$ solution using phenolphthalein indicator.
c) Add 20 ml of N/10 Na_2CO_3 solution and boil again;
d) Cool the contents and filter through Whatman filter paper No. 1 and give 2-3 washings with hot distilled water;
e) Titrate the washings against N/10 HCl using methyl orange indictor;
f) Heat liberated due to production of H_2SO_4 and HNO_3 can be calculated by using the following factors:

1 ml of N/10 $Ba(OH)_2$ solution = 3.60 cal,
and
1 ml of N/10 Na_2CO_3 solution = 1.43 cal

D-5.3.1 Calculations

Nitric acid correction (cal) = $1.43 B (-C)$
Sulphuric acid correction (cal) = $3.6 A [-(B - C)]$

where

A = amount of N/10 $Ba(OH)_2$ solution used (ml);
B = amount of N/10 Na_2CO_3 solution added (ml); and
C = amount of N/10 HCl used (ml).

D-2 CALCULATION OF GROSS ENERGY

Moisture, crude protein, crude fat, crude fibre, and total ash in the *Pangasius* fish feed may be estimated as per the test methods referred in the Table 1.

Nitrogen free extractives (NFE) which represents the available carbohydrate portion of the feed material shall be calculated as below:

Nitrogen free extractives = 100 – (% Moisture + % Crude Protein + % Crude Fat + % Crude Fibre + % Total Ash).

Based on the protein, fat and carbohydrate, the heat of combustion can be calculated and the gross energy equivalent of the test sample can be estimated. The Physiological Fuel Value (gross energy equivalent) for Crude Protein, Crude Fat and Nitrogen free extractives shall be 5.65 kcal/g, 9.45 kcal/g and 4.2 kcal/g, respectively and the Gross Energy of the feed in kcal/kg shall be calculated as given below:

Gross Energy of the feed (kcal/kg) = (crude protein (g/kg) × 5.65) + (crude fat (g/kg) × 9.45) + (NFE (g/kg) × 4.2)

ANNEX E

[*Table 1, Sl No.* (iii)]

ACID HYDROLYSIS

The test for crude fat in finished feeds of *Pangasius* may be carried our as per the method described at Sl No. (iii) of table 1. However, the samples has to undergo acid hydrolysis before subjecting it for fat estimation. The procedure for acid hydrolysis is given below.

E-1 PRINCIPLE

The extruded feed samples forms complexes with fat, protein and carbohydrates. This complexes will not be released in the convention fat estimation by soxhlet method. Hence to break the complexes the feed has to under go acid hydrolysis and then subjected for fat estimation.

E-2 APPARATUS

E-2.1 Oven

E-2.2 Hot Plate

E-2.3 Conical Flask

E-2.4 Balance

E-2.5 Whatman Filter Paper

E-2.6 Stop Watch

E-3 PROCEDURE

About 1 gm sample has to be weighed in a 250 ml conical flask into which 50 ml 3N HCl is added and thoroughly mixed. The flask containing the mixture is gently heated in a hot plate at 90°C for 30 min The heated mixture is filtered using Whatman filter paper on Buchner funnel. The residual acid is removed by rinsing three times the filtered content with distilled water. The filter paper with the feed content has to be carefully removed from Buchner funnel and has to be dried in an oven at 105°C for 3 h. The dried sample is used as such for estimating the fat content using soxhlet method and fat is estimated as described in the Table 1.

ANNEX F
(*Foreword*)

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